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# The Responses of Plant Leaf CO<sub>2</sub>/H<sub>2</sub>O Exchange and Water Use Efficiency to Drought: A Meta-Analysis

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**Abstract:** Persistent drought severely inhibits plant growth and productivity, which negatively affects terrestrial primary productivity worldwide. Therefore, it is important to investigate the impacts of drought on plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and water use efficiency. This study assessed the responses of net photosynthesis (*P<sub>n</sub>*), stomatal conductance (*G<sub>s</sub>*), transpiration (*T<sub>r</sub>*), and instantaneous water use efficiency (WUE) to drought based on a worldwide meta-analysis of 112 published studies. The results demonstrated that drought decreased *P<sub>n</sub>*, *T<sub>r</sub>*, and *G<sub>s</sub>* significantly and differently among different moderators. C<sub>4</sub> plants had smaller *P<sub>n</sub>* reduction than C<sub>3</sub> plants, which gives C<sub>4</sub> plants an advantage in *P<sub>n</sub>*. But their WUE decreased under drought conditions, indicating a great flexibility in C<sub>4</sub> WUE. Annual herbs sacrificed WUE (−6.2%) to maintain efficient *P<sub>n</sub>*. Perennial herbs took a different strategy in response to drought with an increased WUE (25.1%). Deciduous tree species displayed a greater increase in WUE than conifers and evergreen species. Additionally, *G<sub>s</sub>* had a significant correlation with *P<sub>n</sub>* and *T<sub>r</sub>*, but an insignificant correlation with WUE, which could be because WUE is affected by other factors (e.g., air flow, CO<sub>2</sub> concentration, and relative humidity). These findings have significant implications for understanding the worldwide effects of drought on plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and water use efficiency.

**Keywords:** drought stress; meta-analysis; leaf CO<sub>2</sub>/H<sub>2</sub>O exchange; water use efficiency

## 1. Introduction

With a changing climate, drought has been predicted to be more intense and frequent in many regions of the world [1]. Water scarcity has become one of the most adverse environmental factors for plant growth and productivity, limiting terrestrial primary productivity worldwide, especially in the semi-arid regions of the northern hemisphere [2,3]. As a primary resource for plant growth and productivity, water is directly or indirectly involved in all physiological processes. Plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and water use efficiency (WUE, the ratio of net photosynthesis (*P<sub>n</sub>*) to transpiration (*T<sub>r</sub>*)) are important parameters for estimating vegetation productivity and for planning water use in arid and semi-arid areas. An increased WUE can improve plant productivity, and it is necessary for securing environmental sustainable food production in drier environments [4]. Therefore, the effect of drought on leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE is a major issue, especially with a further changing climate [5–7].

The effect of drought on plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE has been intensively studied. According to previous studies, stomata not only control CO<sub>2</sub> flux, but also regulate water flux in plants to reduce water loss and, therefore, affect plant WUE [8–12]. The earliest leaf defense against

desiccation is stomatal closure to avoid excessive water loss [13] since it is much quicker than changes in the root system, leaf area, and chloroplast ultrastructure. Decreasing the supply of CO<sub>2</sub> to mesophyll tissue under drought conditions is widely recognized as the primary effect of drought on carbon assimilation [4,14–16]. During this process, abscisic acid (ABA) reaches the leaves through the transpiration stream for drought-induced root-to-leaf signaling, which induces the stomata closure. Stomata also control *Tr*, which can cool leaf temperatures during drought through water loss [17].

WUE is a key physiological parameter for plant selection. It can be measured at different scales, ranging from instantaneous measurements on the leaf to more integrative measurements at the plant and crop level [18]. Most studies of WUE have used instantaneous measurements of leaf photosynthesis and transpiration to characterize environmental effects [6,19,20], although one study evaluated WUE at the whole-plant level and compared the instantaneous and whole-plant values [18]. In general, WUE has been reported to increase [5,12,21,22] and improve productivity and reduce water stress under drought conditions [7]. As portable equipment for measuring leaf gas exchange rates facilitates the simultaneous measurement of photosynthesis and transpiration, WUE is an early response indicator and an important index for studying the response of plant physiology and ecology to global environmental change.

The responses of plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE to drought differ with the characteristics of the drought event (e.g., the speed, severity, and duration) [23] and with plant endogenous factors (e.g., individual species, growth stage, and plant form) [4,24–26]. Moreover, the results may be different within one species in one experiment [27]. Therefore, there is an urgent need to integrate results across studies to estimate the effects of drought on plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE. In recent years, a number of publications have focused on meta-analysis of plants under stress conditions; this study method is favored because it summarizes and integrates the results from numerous independent experiments while accounting for variability across experiments [28,29]. Meta-analyses have been used to study the responses of plant physiological traits [30] and grain yield [31,32] to drought conditions; and the method has been used to study the responses of plant leaf gas exchange and growth under elevated CO<sub>2</sub> and temperature [33,34]. Researchers have used meta-analysis to explore the central tendency and identify different patterns of leaf gas exchange and water status responses to drought under different moderators (e.g., drought intensity, metabolic CO<sub>2</sub> assimilation, and growth forms) [30]. However, few studies have applied meta-analysis to the worldwide WUE response of plants to an environmental factor.

In this study, we conducted a meta-analysis of plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange (*Pn*, stomatal conductance (*Gs*), and *Tr*) and WUE of plants under drought conditions. Our aim was to determine the responses of plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE to drought. Furthermore, we explored different responses between herb life forms and tree foliage types because foliage types can summarize physiological strategies for water use efficiency and growth patterns in some plant species [35–37]. The objectives of this study were to investigate (1) how drought intensities, photosynthetic pathways (C<sub>3</sub> and C<sub>4</sub>), and plant forms (herbs, shrubs, trees, and lianas) affect the responses of WUE, *Pn*, *Tr*, and *Gs* to drought; (2) the differences between different moderators (e.g., annual herbs (A-herbs) and perennial herbs (P-herbs) and tree foliage types (conifer, deciduous, and evergreen)) under drought; and (3) the relationships between *Gs*, *Pn*, *Tr*, and WUE for the two photosynthetic pathways and four plant forms.

## 2. Methods

### 2.1. Data Collection

The meta-analysis data were collected from peer reviewed journal articles, which were reported or online in the Web of Science and the China Knowledge Resource Integrated Databases. The articles, covering the last 30 years, were selected by the subject term combinations: drought/water stress and water use efficiency/photosynthesis/gas exchange. Finally, there were 112 published papers

selected from more than 600 published related papers. The selected criteria are summarized as follows: (1) the data reported in the papers must be from experiments, including two datasets: control and treatment; (2) all the experiments should report at least one of the observations of plant species (e.g., water use efficiency (WUE), net photosynthesis rate ( $P_n$ ), transpiration ( $Tr$ ) and stomatal conductance ( $G_s$ )). (3) All the observations in the control and treatment groups should have the mean, standard deviation/error and sample size directly extracted from the context, tables and digitized graphs.

Furthermore, datasets were grouped according to (i.) drought intensity or relative soil water content (RSWC), (ii.) photosynthesis pathways, (iii.) plant forms, (iv.) herb life forms and (v.) foliage types. These groups are used as moderators in the meta-analysis to distinguish effective values across and at different moderators, in addition to the overall effects. If the plant forms, foliage type and herb life-form had not been elaborated in the research papers; we would utilize the information from the website of Flora of China: <http://frps.eflora.cn/>. Relevant environment data were also collected: air temperature ( $T_a$ ), air flow ( $A_f$ ),  $CO_2$  concentration ( $CO_2C$ , air relative humidity (RH), leaf temperature ( $T_l$ ), photosynthetically active radiation (PAR), and leaf to air vapor pressure deficit (VPD).

- (i.) Drought intensity is classified into three levels (mild, moderate and severe). Where the drought intensity was not provided in the studies, it would be classified according to the RSWC: mild stress ( $55\% \leq RSWC < 75\%$ ), moderate stress ( $40\% \leq RSWC < 55\%$ ) and severe stress ( $RSWC < 40\%$ ).
- (ii.) Photosynthesis pathways include the  $C_3$  and  $C_4$  photosynthetic pathway ( $C_3$  and  $C_4$  plants), but we did not take crassulacean acid metabolism (CAM) photosynthesis into consideration.
- (iii.) Plant forms (e.g., trees, shrubs, lianas and herbs) have widely different mechanical architecture, which can also vary phenotypically with the environment.
- (iv.) Herb life forms: annual and perennial herbs. A-herbs and P-herbs are short for annual herbs and perennial herbs, respectively.
- (v.) Foliage types: there are three different foliage types, including needle-leaved evergreen conifers (conifer), evergreen broadleaves (evergreen) and deciduous broadleaves (deciduous).

It should be noted that a total of 124 plant species (92 genera in 44 families) are recorded, and 1690 observations are selected from 112 published papers, including 521 observations of  $P_n$ , 404 observations of  $Tr$ , 356 observations of  $G_s$  and 409 observations of WUE.

## 2.2. Meta-Analysis

The effect size for each observation is calculated as the response ratio ( $LnR$ ) to represent the magnitude of the responses of plant leaf  $CO_2/H_2O$  exchange and WUE to drought:

$$LnR = \ln(X_e/X_c) = \ln(X_e) - \ln(X_c) \quad (1)$$

where  $X_e$  and  $X_c$  are the mean response values of each individual observation in the treatment and control groups. The variance of the response ratio ( $LnR$ ) was calculated:

$$v = S_e^2/(n_e X_e^2) + S_c^2/(n_c X_c^2) \quad (2)$$

where  $S_e$  and  $S_c$  are the standard deviations for the treatment and control groups;  $n_e$  and  $n_c$  are the sample sizes for the treatment and control groups;  $X_e$  and  $X_c$  are the same indicators as in Equation (1). In order to improve the accuracy of  $LnR$  and reduce its variability, the mean weighted response ratio ( $LnR^+$ ) is calculated from  $LnR$ :

$$LnR^+ = \sum(LnR_i \times w_i) / \sum(w_i) \quad (3)$$

where  $i$  is the categorical subdivision for group analysis; the reciprocal of the variance ( $w_i$ ) is the weight of each  $LnR$  and calculated as follows:

$$w_i = 1/v \quad (4)$$

The meta-analyses are performed using METAWIN 2.0 (Sinauer Associates, Sunderland, UK) [38]. The effect is analyzed by the random effect model. The mean effect size of each categorical subdivision is calculated, and if the 95% confidence intervals (CI) of  $LnR^+$  do not overlap with zero, this means that the drought has a significant promoting or inhibiting effect on plant leaf  $CO_2/H_2O$  exchange and WUE. The bigger the value is, the greater the influence of drought on the plants.

Then, the  $LnR^+$  is reported as the percent change ( $D$ , %), which is more visible than  $LnR^+$  and calculated as follows:

$$D(\%) = (e^{LnR^+} - 1) \times 100\% \quad (5)$$

Regression analyses are performed to establish the relationships between  $G_s$ ,  $P_n$ ,  $Tr$  and WUE with environmental factors under drought in all plant species, two photosynthesis pathways and four plant forms. The  $G_s$ ,  $P_n$ ,  $Tr$  and WUE for this regression analysis are dependent and measured from the same leaf at the same time. The  $LnR$  is effect size for  $G_s$ ,  $P_n$ ,  $Tr$  and WUE and calculated as Equation (1). Figures are performed with Origin Pro 9.0 software (OriginLab Corporation, Northampton, MA, USA) with Microsoft Windows.

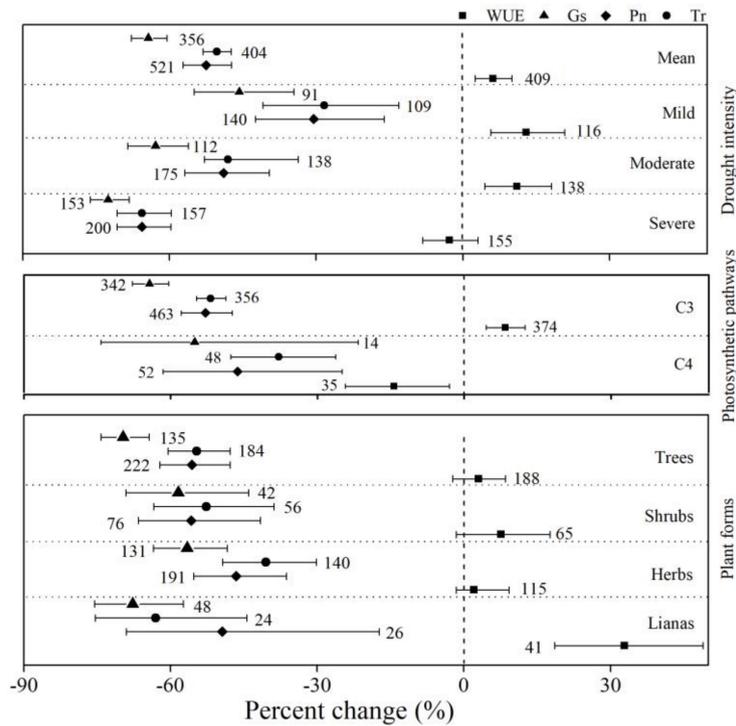
### 3. Results

#### 3.1. Effects of Drought on Plant Leaf $CO_2/H_2O$ Exchange and WUE

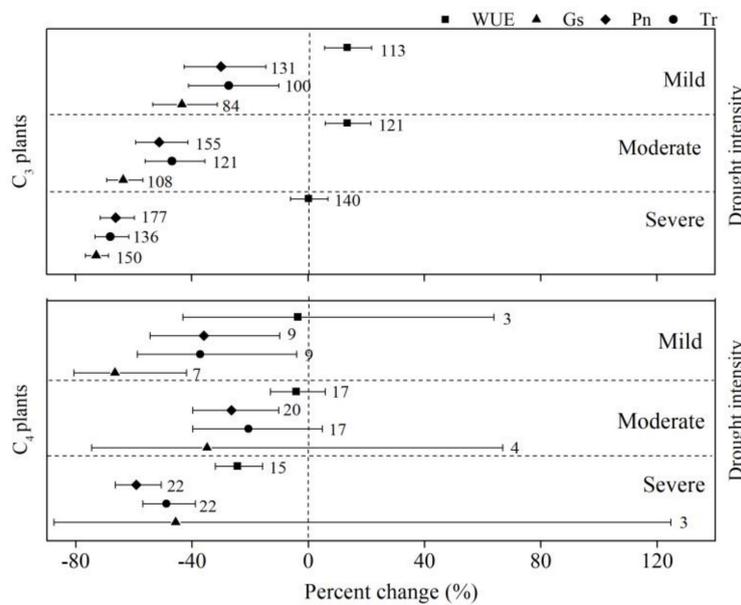
As shown in Figure 1, drought showed a significant, adverse influence on plant leaf  $CO_2/H_2O$  exchange. Across all studies, drought decreased  $P_n$ ,  $Tr$ , and  $G_s$  by 52.5%, 50.3%, and 64.3%, respectively, but increased WUE by 6.2% (Figure 1).  $G_s$  decreased more than  $P_n$  and  $Tr$  in all studies, regardless of the different groups. Drought decreased the  $P_n$ ,  $Tr$ , and  $G_s$  of  $C_3$  plants by 52.9%, 51.8%, and 64.3%, respectively, which were greater than the decreases in  $P_n$ ,  $Tr$ , and  $G_s$  for  $C_4$  plants (−46.3%, −37.9%, and −55.1%, respectively). However, drought increased the WUE in  $C_3$  plants by 8.4% and decreased the  $C_4$  plants' WUE by 14.3%. Drought intensity showed an adverse influence on plant leaf  $CO_2/H_2O$  exchange, with the most adverse effects appearing under severe stress. The WUE increased by 13.0% and 11.1% under mild and moderate stress, indicating a significant promoting effect. Severe stress decreased WUE by 2.7%, although the 95% CI overlapped with zero. Drought decreased the  $P_n$ ,  $Tr$ , and  $G_s$  of herbs by 46.6%, 40.5%, and 56.6%, respectively (Figure 1), and this was the lowest percent change among the four plant forms. The percent change of WUE for trees, shrubs, and herbs overlapped with zero, showing an insignificant effect of drought. The percent change of WUE was the highest for lianas (32.9%), indicating that drought had the greatest promoting effect on lianas among the four plant forms.

#### 3.2. Effects of Drought on Plant Leaf $CO_2/H_2O$ Exchange and WUE in $C_3$ and $C_4$ Plants

The results showed that the changes in  $P_n$ ,  $Tr$ , and  $G_s$  in  $C_3$  plants were smaller than for  $C_4$  plants under mild stress, but larger than  $C_4$  plants under moderate and severe stress (Figure 2). The percent change of WUE for  $C_3$  plants was the same (13.5%) under mild and moderate stress, but the percent change overlapped with zero under severe stress (Figure 2). The WUE of  $C_4$  plants decreased by 3.5%, 4.1%, and 24.3% under mild, moderate, and severe stress, respectively, with the 95% CI overlapping with zero under mild and moderate stress.



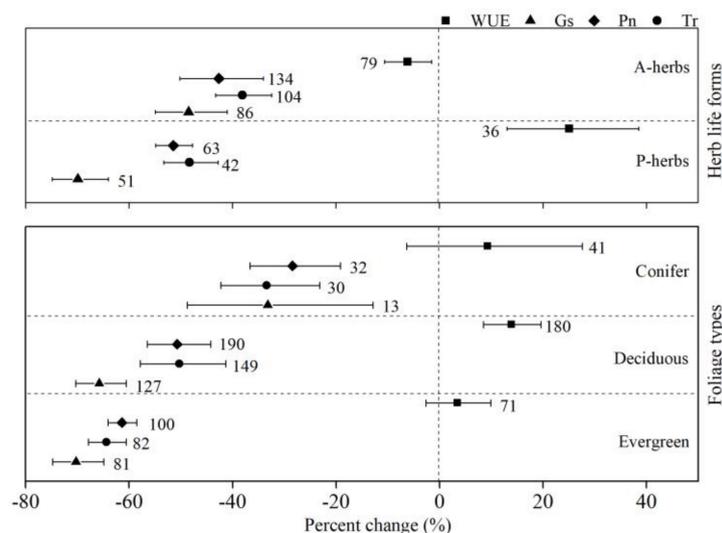
**Figure 1.** Percent change of net photosynthesis (*Pn*), stomatal conductance (*Gs*), transpiration (*Tr*), and water-use efficiency (*WUE*) under three different moderators: photosynthetic pathway (*C*<sub>3</sub> and *C*<sub>4</sub> plants), drought stress (mild, moderate, and severe), and plant forms (trees, shrubs, herbs, and lianas). Numbers near the symbols specify the number of data points, and the error bars show the 95% confidence interval (CI).



**Figure 2.** Comparison of the percent change of net photosynthesis (*Pn*), stomatal conductance (*Gs*), transpiration (*Tr*), and water-use efficiency (*WUE*) for photosynthesis pathways (*C*<sub>3</sub> and *C*<sub>4</sub> plants) under different drought intensities (mild, moderate, and severe). Numbers near the symbols specify the number of data points, and the error bars show the 95% CI.

### 3.3. Effects of Drought on Plant Leaf $\text{CO}_2/\text{H}_2\text{O}$ Exchange and WUE of Herb Life Forms and Tree Foliage Types

Herb life forms showed different responses to drought. P-herbs showed larger changes in  $P_n$ ,  $Tr$ , and  $G_s$  ( $-51.4\%$ ,  $-48.3\%$ , and  $-69.9\%$ , respectively) than A-herbs ( $-42.6\%$ ,  $-38.1\%$ , and  $-48.5\%$ , respectively). Drought increased the P-herbs' WUE by 25.1%, but decreased the A-herbs' WUE by 6.2%. Drought decreased  $P_n$ ,  $Tr$ , and  $G_s$  of conifer plants by  $-28.4\%$ ,  $-33.4\%$ , and  $-33.2\%$ , respectively, the smallest reduction of the foliage types. Evergreen plants had the greatest decrease in  $P_n$ ,  $Tr$ , and  $G_s$  ( $-61.4\%$ ,  $-64.4\%$ , and  $-70.3\%$ , respectively). Drought had the greatest promoting effect on the WUE of deciduous plants (13.9%). The 95% CIs overlapped for the percent changes of WUE for conifers and evergreen plants and the intervals overlapped with zero (Figure 3).



**Figure 3.** Percent changes of net photosynthesis ( $P_n$ ), stomatal conductance ( $G_s$ ), transpiration ( $Tr$ ), and water-use efficiency (WUE) for herb life forms (A-herbs and P-herbs) and tree foliage types (conifer, deciduous, and evergreen). Numbers near the symbols specify the number of data points, and the error bars show the 95% CI.

### 3.4. Relationship of $G_s$ , $P_n$ , $Tr$ and WUE With Environmental Factors

The results showed that there was a significant positive correlation between  $G_s$  and  $P_n$  ( $R^2 = 0.5087$ ,  $P < 0.01$ ) across all plant types (Table 1). There was a positive correlation between  $G_s$  and  $P_n$  in both  $C_3$  plants ( $R^2 = 0.4968$ ,  $P < 0.01$ ) and  $C_4$  plants ( $R^2 = 0.7545$ ,  $P < 0.01$ ).  $G_s$  was correlated with  $P_n$  in trees, shrubs, herbs, and lianas (49.21%, 53.3%, 50.46%, and 72.1%, respectively ( $P < 0.01$ )) (Table 1). Our results showed a positive correlation between  $G_s$  and  $Tr$  at different moderators ( $P < 0.01$ ).  $G_s$  was correlated with  $Tr$  for all species,  $C_3$  plants, and  $C_4$  plants (72.06%, 77.94%, and 76.57%, respectively).  $G_s$  was also correlated with  $Tr$  for trees, shrubs, herbs, and lianas (90.48%, 53.09%, 48.29%, and 87.37%, respectively).  $G_s$  and WUE had an insignificant negative correlation in different groups, although shrubs had a positive correlation.  $G_s$  was correlated with WUE for all plants (16.15% ( $P < 0.01$ )), but only 1.04% ( $P = 0.12$ ) and 15.47% ( $P = 0.16$ ) correlations for  $C_3$  and  $C_4$  plants, respectively. The correlation between  $G_s$  and WUE was 3.49% ( $P = 0.076$ ), 13.67% ( $P = 0.783$ ), 23.79%, and 52.7% ( $P < 0.01$ ) for trees, shrubs, herbs, and lianas, respectively.

**Table 1.** The relationships between the  $G_s$  response ratio and other factors ( $P_n$ ,  $Tr$ , WUE).

Moderators	Fitting	$R^2$	$P$	N
All species	$P_n = 0.6846G_s - 0.1801$	0.5087	<0.01	333
	$Tr = 0.7937G_s - 0.0862$	0.7206	<0.01	361
	$WUE = -0.2354G_s - 0.1115$	0.1615	<0.01	350
$C_3$ plants	$P_n = 0.7378G_s - 0.1482$	0.4968	<0.01	319
	$Tr = 0.8249G_s - 0.0650$	0.7794	<0.01	347
	$WUE = -0.0858G_s - 0.0788$	0.0104	0.12	347
$C_4$ plants	$P_n = 0.7744G_s - 0.0326$	0.7545	<0.01	25
	$Tr = 0.6699G_s - 0.1391$	0.7657	<0.01	25
	$WUE = -0.1723G_s - 0.0016$	0.1547	0.16	14
Trees	$P_n = 0.8067G_s - 0.0904$	0.4921	<0.01	132
	$Tr = 0.9004G_s + 0.0258$	0.9048	<0.01	132
	$WUE = -0.1819G_s - 0.2457$	0.0349	0.08	130
Shrubs	$P_n = 0.6377G_s - 0.4584$	0.5330	<0.01	43
	$Tr = 0.6527G_s - 0.4181$	0.5309	<0.01	43
	$WUE = 0.1487G_s - 0.0104$	0.1367	0.78	40
Herbs	$P_n = 0.6819G_s - 0.1525$	0.5046	<0.01	132
	$Tr = 0.6297G_s - 0.1453$	0.4829	<0.01	132
	$WUE = -0.3579G_s - 0.1480$	0.2379	<0.01	116
Lianas	$P_n = 0.4848G_s - 0.0795$	0.7207	<0.01	24
	$Tr = 0.8601G_s + 0.0006$	0.8737	<0.01	24
	$WUE = -0.4282G_s - 0.1900$	0.527	<0.01	41

$G_s$ : Stomatal conductance;  $P_n$ : Net photosynthesis;  $Tr$ : Transpiration rate; WUE: Water-use efficiency; N: Number of samples.

Pearson correlation analysis showed that the response ratios of the  $P_n$  to drought did not exhibit any significant correlation with air flow ( $Af$ ),  $CO_2$  concentration ( $CO_2C$ ), air relative humidity (RH), leaf temperature ( $T_l$ ), photosynthetically active radiation (PAR) or leaf to air vapor pressure deficit (VPD) (Table 2), but a significant positive with air temperature ( $T_a$ ) ( $P < 0.05$ ). The response ratios of  $Tr$  to drought was observed a significant negative relationship with  $Af$  and a positive relationship with RH ( $P < 0.05$ ). Significant positive relationships were observed between the response ratios of  $G_s$  to drought with  $CO_2C$ , RH ( $P < 0.01$ ) and  $T_l$  ( $P < 0.05$ ). WUE had a significant positive relationship with  $Af$  ( $P < 0.01$ ), but significant negative relationships with  $CO_2C$  ( $P < 0.05$ ) and RH ( $P < 0.01$ ).

**Table 2.** Pearson correlation coefficients between the  $P_n$ ,  $Tr$ , WUE response ratios and other factors.

Categorical Type		$T_a$	$Af$	$CO_2C$	RH	$T_l$	PAR	VPD
All plants	$P_n$	0.19*	0.11	0.07	0.07	0.09	0.11	0.05
	$Tr$	0.21	-0.46**	0.08	0.23*	0.14	0.10	0.01
	$G_s$	0.08	-0.22	0.26**	0.38**	0.24*	0.09	0.10
	WUE	0.09	0.38**	-0.14*	-0.31**	-0.19	0.09	0.09
	N	219	70	309	125	140	394	83

$T_a$ : air temperature;  $Af$ : air flow;  $CO_2C$ :  $CO_2$  concentration; RH: air relative humidity;  $T_l$ : leaf temperature; PAR: photosynthetically active radiation; VPD: leaf to air vapor pressure deficit; N: number of samples; \*  $< 0.05$ ; \*\*  $< 0.01$ .

#### 4. Discussion

Our meta-analysis was the first comprehensive overview of the effects of drought on plant leaf  $CO_2/H_2O$  exchange and WUE. We found that drought strongly inhibited plant leaf  $CO_2/H_2O$  exchange (Figure 1), and the overall inhibitory effect increased with increasing drought stress. We found that  $G_s$  decreased more than  $P_n$  and  $Tr$  across all studies and in different groups, which was consistent with previous meta-analysis results [30]. The governing of stomata opening and closure is crucial for terrestrial plants (particularly  $C_3$  plants) to balance carbon acquisition and water loss by transpiration [39]. Decreased transpiration caused by the regulating mechanism to reduce  $G_s$  during

drought conditions may be an adaptive behavior to protect the conducting system against hydraulic failure while restricting the entry of CO<sub>2</sub> [40,41].

The changes in  $P_n$  and  $Tr$  controlled by  $G_s$  caused changes in WUE under drought condition. The unexpected finding was that drought increased WUE, to some extent, across all studies, and the promoting effect was found under mild and moderate stress. The decrease in  $P_n$ ,  $Tr$ , and  $G_s$  and concurrent increase in WUE in response to decreased soil moisture in species is common [5,22,42]. Theoretically, WUE may be improved by partial closure of stomata so that the intercellular CO<sub>2</sub> concentration is sufficient for saturation of  $P_n$  while the rate of water loss ( $Tr$ ) can be significantly lowered under mild and moderate stress [21].

Herbs had a smaller decrease in  $G_s$  than trees under drought conditions, which supports the hypothesis that herbs adapt poorly to drought conditions [30] due to their high  $Tr$  and significant water loss in the absence of a large root system. Trees have higher drought resistance than other plants due to their lower  $Tr$  and large root systems, and this study confirmed that the deep rooting habit of trees enables them to extract water from depths of up to 8m, reducing sensitivity to surface soil water deficiency [22].

The change in WUE in trees, shrubs, herbs, and lianas were significantly different. Lianas had the largest change in WUE followed by shrubs, trees, and herbs. Drought had a marked improvement on lianas' WUE, but the effect was not significant for trees, shrubs, and herbs (Figure 1). The  $G_s$  of lianas and trees decreased more than shrubs and herbs, indicating their superior adaptation to drought, as they benefit from strong stomatal control to avoid excessive water loss during water deficits [43]. Lianas had smaller reductions in  $P_n$  and  $G_s$  (Figure 1) than trees, which is consistent with previous research that they have better access to deep soil water than co-occurring trees [43]. Lianas had the largest increase in WUE, suggesting this plant type has an advantage over trees due to a smaller proportional decline in  $P_n$  and a larger decline in  $Tr$  during drought.

The responses of plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE to drought can vary due to other factors (i.e., species and stress intensity) [14]. Therefore, we identified the responses of plant leaf CO<sub>2</sub>/H<sub>2</sub>O exchange and WUE to drought as affected by the following combination moderators: drought intensity and photosynthetic pathways, herbs life forms, and foliage types.

#### 4.1. Photosynthetic Pathways

The leaf CO<sub>2</sub>/H<sub>2</sub>O exchange in C<sub>3</sub> plants was more influenced by drought than C<sub>4</sub> plants, showing significant and larger reductions in  $P_n$ ,  $Tr$ , and  $G_s$ . This suggested that C<sub>4</sub> plants have higher carbon sequestration under drought conditions. These results are consistent with previous research that C<sub>4</sub> plants have an advantages in  $P_n$  in drought conditions compared with C<sub>3</sub> plants [30,44,45] because the C<sub>4</sub> CO<sub>2</sub>-concentrating mechanism offers C<sub>4</sub> photosynthesis a greater buffering capacity against CO<sub>2</sub> shortages due to partial stomatal closure under water stress [46]. However, drought increased C<sub>3</sub> WUE overall, but tended to decrease C<sub>4</sub> WUE (Figure 1). The result seems to contradict the advantage C<sub>4</sub> plants have over C<sub>3</sub> plants in WUE under drought conditions [5,47]. We observed reductions in C<sub>4</sub> WUE, especially for severely stressed plants (e.g., maize (*Zea mays*) [27] and some C<sub>4</sub> grass species). Severe stress causes notable reductions in the WUE of maize in the vegetative and reproductive stages, whereas moderate stress results in significantly decreased WUE in the later growth stages [27]. Percy et al. [48] compared the photosynthetic type of plants limited by water deficiency, and the advantage of the C<sub>4</sub> plant (*Amaranthus retroflexus*) over the C<sub>3</sub> plant (*Chenopodium album*) displayed in good water conditions disappeared in drought conditions. These results indicated that drought is not the precondition for the dominance of C<sub>4</sub> plants in many plant communities [49]. The C<sub>4</sub> WUE is complex; the reduction in leaf  $P_n$  and  $Tr$  under water stress varies with the intensity and time of the drought, the plant species, and a variety of environmental factors. Some C<sub>4</sub> plant species (e.g., *Spartina alterniflora*) have increased WUE to adapt to severe drought conditions [50]. This indicates that C<sub>4</sub> plants have flexible WUE. Drought does, to a certain extent, increase the C<sub>3</sub> plants' WUE, but the WUE of C<sub>3</sub> plants is not necessarily higher than that of C<sub>4</sub> plants under drought

conditions. Research has shown that  $C_4$  plants have a greater WUE than  $C_3$  plants in desert areas [51]. For example, a previous study found that the WUE of  $C_4$  plants was significantly higher than that of  $C_3$  species under drought stress in *Flaveria* species [5]. *Atriples confertifolia* ( $C_4$ ) has been found to have significantly higher WUE in summer drought conditions than *Ceratoides lanata* ( $C_3$ ), although they had similar ecotypes in similar environmental conditions [52].

#### 4.2. Herbs Life Forms

Our study found that the lifespan of herbs (A-herbs and P-herbs) has an apparent impact on the responses of leaf  $CO_2/H_2O$  exchange and WUE to drought. P-herbs' percent changes of  $Pn$ ,  $Tr$ , and  $G_s$  (mainly alfalfa and grazing grass) were larger than those of A-herbs (mainly crops such as rice, maize, barley, and wheat). Also, drought decreased the WUE in A-herbs, but increased the WUE in P-herbs, showing an opposite effect (Figure 3). These findings suggest that perennial herb species have a different drought response than annual species. A large decline in the  $G_s$  of P-herbs reduced an already low  $Tr$  to avoid dehydration. Therefore, leaves and stems of P-herbs are more vulnerable to dieback from high temperatures destroying their photosynthetic mechanisms. Other plant parts (e.g., roots, caudex, and various types of underground stems) survive under or close to the ground in drought conditions. Studies have suggested that dehydration avoidance and dormancy are perennial herbs' main strategies for survival in summer droughts in Mediterranean areas [53]. Furthermore, drought survival can impact WUE through plant mortality and stand recovery after drought. In drought conditions, annual herbs try to maintain their  $Pn$  to finish their reproductive growth through higher  $G_s$  of  $CO_2$  and higher water flux exchange than perennial herbs. This causes a reduction in annual herb WUE. Research has confirmed that A-herbs have a better WUE than perennial herbs in desert areas [51].

#### 4.3. Foliage Types

We found that the leaf  $CO_2/H_2O$  exchange in conifers, evergreens, and deciduous plants decreased markedly under drought stress, but the percent changes were different. Evergreen trees had the largest changes in  $Pn$ ,  $Tr$ , and  $G_s$  under drought stress (Figure 3), and conifers had the smallest change. We expected that the different responses between the three foliage types may be caused by different leaf functional anatomy (e.g., leaf area, thickness, specific leaf area, density, and stomatal morphology) [54,55], which is supported by a previous study that showed a tight coordination between leaf morphology and photosynthetic properties in water deficit [56]. These results have been found in several tree species that down-regulate photosynthesis during drought, suggesting that species with different leaf habits differ with respect to morphological, chemical, and physiological traits [17]. We found that all percent changes in WUE for coniferous, deciduous, and evergreen plants were greater than zero, especially in the deciduous species. This suggests that stomata closure in response to  $H_2O$  flux is more sensitive than the response to carbon flux [22]. In addition, evergreen species are thought to have leaf traits that cause lower photosynthesis and transpiration rates, which reduces WUE [57]. Evergreen species may extend their leaf lifespans by protecting their photosynthetic machinery from overheating through evaporative cooling [17,57]. However, we found that deciduous species increased WUE under drought conditions with a smaller reduction in  $Tr$  than  $Pn$ . In addition to losing a portion of their leaves, deciduous species avoid high temperature damage by minimizing water loss from their remaining leaves [58].

#### 4.4. Relationship of $G_s$ , $Pn$ , $Tr$ and WUE with Environmental Factors

Based on the fitted results of  $G_s$  with  $Pn$  and  $Tr$  in this study and previous research [30],  $G_s$  showed significant relationship with  $Pn$  and  $Tr$  in  $C_3$  and  $C_4$  plants and the four plant forms (trees, shrubs, herbs, and lianas) on a global scale. The results indicated that stomata play a more important role in controlling transpiration rates than photosynthesis rates [30]. Many studies have determined that stomatal limitation is the dominant control of  $Pn$  and  $Tr$  reduction under drought stress [4,13,16,59,60].

Unexpectedly,  $G_s$  had a low explanation rate (16.15%) across all studies, suggesting it did not explain WUE results (Table 1). It also had low explanation rates for photosynthetic pathways ( $C_3$  and  $C_4$  plants) and plant forms (trees, shrubs, herbs, and lianas). However, our study found a negative correlation between WUE and  $G_s$  (Table 1), which is supported by previous reports that stress-induced reduction in  $G_s$  has a greater effect on  $Tr$  than  $P_n$  [61]. Therefore, WUE is a complex index that is affected by  $G_s$  and other factors. The Pearson correlation analysis showed that the response ratios of WUE to drought had a significant correlation with the air flow rate (Af), and a significant, negative relationship with  $CO_2$  concentration and relative humidity (RH) among all plants (Table 2). Previous research has suggested that WUE is in part driven by changes in environmental conditions, such as relative humidity in the air and temperature [62], and the leaf position in the canopy can also have a marked effect on instantaneous leaf WUE [18].

## 5. Conclusions

In this meta-analysis study, we found that drought decreased  $P_n$ ,  $Tr$ , and  $G_s$  significantly and differently among different moderators. The WUE increase of lianas was greater than trees, shrubs, and herbs.  $C_4$  plants had a smaller  $P_n$  reduction than  $C_3$  plants, which gives  $C_4$  plants an advantage in  $P_n$ . But their WUE decreased under drought conditions, indicating a great flexibility in  $C_4$  WUE. We found that annual herbs sacrificed high WUE to maintain efficient  $P_n$  to finish their reproductive growth in their short life cycle. Perennial herb species have a different strategy in response to drought with an increased WUE (25.1%). Deciduous tree species displayed a greater increase in WUE than conifer and evergreen species. Additionally, we found that  $G_s$  was significantly positively correlated with  $P_n$  and  $Tr$ , but was insignificantly negatively correlated with WUE, which could explain the stress-induced reduction in  $G_s$  having a greater effect on  $Tr$  than  $P_n$ . WUE is a complex index that not only affected by  $G_s$ , but also by other factors (e.g., air flow,  $CO_2$  concentration, and relative humidity). These findings have significant implications for understanding the worldwide effects of drought on plant leaf  $CO_2/H_2O$  exchange and water use efficiency.

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